PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
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Crystal Plaza 2
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ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 February 1999 (24.02.99)	
International application No. PCT/GB98/01893	**Applicant's or agent's file reference** SMK/CP5710306
International filing date (day/month/year) 29 June 1998 (29.06.98)	**Priority date** (day/month/year) 27 June 1997 (27.06.97)
Applicant ARCHER, John, Anthony, Charles et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 January 1999 (18.01.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

H. Zhou

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SMK/CP5710306		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/01893	29/06/1998	27/06/1997
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant CAMBRIDGE UNIVERSITY TECHNICAL SERVICES ....et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/01/1999	13. 10. 99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Luzzatto, E Telephone No. +49 89 2399 8169

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/01893

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

2-21,23-42	as originally filed	
1,1b,22	with telefax of	24/08/1999

Claims, No.:

1-45	with telefax of	24/08/1999

Drawings, sheets:

1/16-16/16	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 11,27,28,34.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 11,27,28,34 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10,12-24,33,37,40-45
	No:	Claims	25,26,29-32,35,36,38,39
Inventive step (IS)	Yes:	Claims	5,7,37,40-43
	No:	Claims	1-4,6,8-10,12-26,29-33,35,36,38,39,44,45
Industrial applicability (IA)	Yes:	Claims	1-45
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

PART III

No examination of the subject-matter of claims 11, 27, 28 and 34 as to novelty and inventive step was carried out for the reasons given in part V and VIII hereinbelow.

PART V

1) The subject-matter of claims 25, 26, 29-32, 35-39 lacks novelty (Art. 33(2) PCT).

1.1) Li et al. (J. Bacteriol., 6409-18,1996) (D1) disclose the Rhodococccus *dsz* promoter and regulatory sequences associated therewith. The promoter was cloned and directed the expression of a reporter gene in a Rhodococcus strain. Mutagenesis studies were also carried out (see abstract, "materials and methods", table 1 and discussion). In view of the fact that:

a) a product is not rendered novel by the method by which it can be obtained but only by its intrinsic characteristics and that

b) the promoter disclosed in D1 appears to have the same characteristic as a promoter which could be obtained by the method to which claim 1 relates,

The term "biosensor" does not limit the scope of the claim to transformed microorganisms actually **used** as biosensors but it only relates to transformed microorganisms **suitable** for use as biosensors, such as those disclosed in D1.
As to the feature of claim 1 concerning lack of catabolic repression, it would appear that the mutants described in D1 (p. 6411, col. 2, l. 34-p. 6412, l. 4) lack catabolic repression and hence fully fall within the scope of claims 25, 26, 29-32, 35-39, which thus lack novelty (Art 33(2) PCT).
Moreover, it appears that the fact that a strain lacks catabolic repression is not necessarily linked to the sequence of the isolated promoter/operon proteins. From D1 it appears clearly that mutations within the promoter sequence can lead to the loss of catabolic repression; however, D1 also discloses mutations lying outside the promoter sequence (in a non coding region) which cause loss of catabolic repression (abstract); it also appears (see the description, p. 9, l. 16-32) that other mutations could cause such a loss, e.g. a mutation in a sigma factor. Hence, the conclusion to be drawn is that the absence of catabolic repression in a strain certainly characterises the strain, but does not however necessarily characterise the **isolated** DNA encoding an inducible promoter. For this reason, the following

documents take away novelty from some of the aforecited claims:

1.2) EP-A-759474 (D2) discloses promoters and genetic constructs which appear to anticipate the subject-matter of claims 26 (see p. 2, l. 34-53 and ex. 1).

1.3) Denome et al. (Appl. Environm. Microbiol., 2837-43, 1993) (D3), cited in view of the Guidelines, C-VI, 7.24, teaches Rhodococcus nucleic acid sequences which are capable of site-specific integration and confer the DBT desulfurisation phenotype (see the abstract). It thus anticipates the subject-matter of claims 26, 35-39.

2) In view of the foregoing, it has to be assumed that claim 1 relates to sequences isolated from strains lacking catabolic repression. The said sequences as such, however, are not so characterised. Hence, the subject-matter of claims **1-4, 6, 8-24, 33, 44 and 45** lacks an inventive step (Art. 33(3) PCT).

2.1) D4 (Omori et al., Appl. Environm. Microbiol., 911-15, 1992)(Guid., C-VI, 7.24) discloses a method for the isolation of a Corynebaterium strain (which was later identified as belonging to the Rhodococcus genus, see D4a, Omori et al., Biosci. Biotech. Biochem., 59(7), 1195-98, 95) which specifically metabolises DBT (see the abstract and the first two paragraphs of "Materials and Methods").
Starting form D4, the technical problem underlying the present application can be seen in the identification and isolation of the DNA which is responsible for the substrate specificity.
Such a procedural step is well established in the art, as shown e.g. by D1, which characterises the genes of the previously isolated Rhodoccoccus strain IGTS8 (see passages cited hereinabove) or WO-A-9215687 (D5), which teaches the same method as applied to other microorganisms: the microorganisms are then used as biosensors (see p. 19, l. 26-p. 20, l. 12, claims).
Hence the subject-matter of claims **1-4** lacks an inventive step.

2.2) The subject-matter of claims 8-10, 12-24 and 33 does not appear to involve an inventive step since it forms part of the general knowledge of the skilled person.

a) As to claim 8, in view of the fact that the use of e.g. Tween (which is the detergent

whose use is exemplified in the description) is current practice in the art, an inventive step could only have been acknowledged if the alleged advantages of its use had been supported by e.g. comparative examples.

b) The Applicant submitted data which could support the presence of an inventive step for the subject-matter of claim 9, **insofar as related to the use of the recO mutation.** No such data are available for the other mutations. The subject-matter of claim 9, hence, lacks an inventive step.

c) As to claims 22 and 23, an inventive step could only have been acknowledged if the Applicant had shown that the selected elements and plasmids exhibit an unexpected effect.

d) As to claim 33, the genes to which it relates are commonly used as reporter genes (see e.g. D1).

2.3) As to claim 44:
as explained in part V(1) above, the term biosensor is not limiting; hence, within the scope of the claim, which is to be construed as a purely product-related claim, since the expresion "for performing...." is not limiting (Guidelines, C-III, 4.8), fall any reagent kits comprising a suitably modified mycolic acid bacteria and **any** reagent for performing the method of claims 40-43. The reagent could for instance be the culture medium: D1 (see above) therefore renders obvious the subject-matter of claim 44, insofar as the method therein disclosed comprises a suitable transformed Rhodococcus (see item 1.1 above) and the said reagent and in view of the fact that packaging known reagents which are used in a known or obvious method does not involve an inventive step.

2.4) Claim 45 lacks an inventive step (see also part VIII hereinbelow).
It encompasses **any** (the expression "for performing" is not limiting, see Guidelines, C-III, 4.8) kit comprising a non-ionic detergent and **any** shuttle vector comprising e.g. a replicon for E. Coli and a sequence encoding an antibiotic marker gene: however, not all shuttle vectors belonging to this broad class would contribute to the solution of the technical problem underlying the present

invention. The scope of the claim should have thus been limited to those kit components actually helping solve the said problem.

3) Claims **5, 7, 37, 40-43** meet the requirements of Art. 33 PCT

3.1) The subject-matter of claims 5 and 7 is not disclosed or suggested by any of the available documents. No document discloses the use of a medium as that claimed in claim 5 or 7. Moreover, the advantages which the use of the said medium have (see p. 8, l. 4-26) further support the presence of an inventive step.
The subject-matter of claims 5 and 7 appears thus to meet the requirements of Art. 33 PCT.

3.3) The subject-matter of claims 37 and 40-43 is also considered to involve an inventive step in view of the fact that the mycolic acid bacterium used in the claimed method is characterised by the lack of catabolic repression.
Concerning the subject-matter of claims 40-43, the closest prior art is identified in D5 (WO-A-9215687) which teaches the production of microorganism-based biosensors (see p. 9, l. 8-p. 10, l. 10, claims). The microorganisms whose use is disclosed in D5 belong to the genus Alcaligenes, which is not mentioned as belonging to the group of mycolic bacteria.
Hence the technical problem underlying the present invention can be seen in the preparation of biosensors for the detection of compounds different from those detectable with the biosensors disclosed in D5.
Although the characteristics of mycolic acid bacteria such as Rhodococcus as well as the genes involved in the metabolic pathways were well known (see e.g. D1 or D2 or D3) the skilled person would not have had any indication as to the use of mycolic acid bacteria strains lacking catabolic repression. The advantages thereof (p. 9, l.33-p. 10, l. 31) further support the presence of an inventive step.
The method of transforming a cell to which claim 37 relates allows the production of a mycolic acid strain which lacks catabolic repression and which incorporates a heterologous gene.

3.4) An assessment as to inventiveness for the subject-matter of claims 11, 27 and 28 is not possible due to the lack of clarity of the said claims (see Part VIII(5) hereinbelow). The IPEA is however of the opinion that, if the said claims had

related clearly and unambiguously to the specific sequence of the R. Corallina ohp operon as given e.g. in fig. 4 an inventive step could have been possibly acknowledged. The said sequence is neither taught nor rendered obvious by any of the available documents. Moreover, even if the description does not disclose whether the R. corallina ohp operon lacks catabolic repression or not, the said sequence would in any case allow the skilled person to effectively screen mycolic acid bacteria libraries (see p. 11, l. 2-7 of the description).

4) No examination as to novelty and inventive step can be carried out concerning the subject-matter of claim 34 due to the absence of the technical features of pJP7, both in the claims and in the description (see Part VIII, point 3 hereinbelow).

PART VIII

1) Claim 45 lacks clarity (Art. 6 PCT) since the vector to which it relates is only defined by reference to method claims which however do not provide a sufficient characterisation of the said vector. To overcome this clarity objection, the features of the vector should have been introduced into the claim.

2) Claim 39 lacks concisesness and clarity (Art. 6 PCT) because the reference to claim 32-34 is redundant, since the method of claim 38, by referring to claim 35, encompassess the transformation of a host cell with the vectors of claims 32-34. The lack of clarity arises because claim 39 could be interpreted as indicating that transforming the cell with the vectors of claims 32-34 is a different process to that to which claim 35 relates.

3) Claims 24 and 34 lack clarity (Art. 6 PCT) due to the use of terms (e.g. pJP7) which are but internal designations devoid of any technical meaning. To overcome this objection, the claims should have been amended by introducing into their subject-matter the technical features of the plasmids and vector respectively.

4) The Applicant stated that *mac* is but another designation of *ohp*. However, from the passage on p. 12, l. 16-31 it would appear that they are indeed different.

5) Claims 11, 27 and 28 lack clarity (Art. 6 PCT) in view of the absence of any reference to a specific sequence. The mere designation of a gene or an operon does not suffice to technically characterise the subject--matter of the claims

BIOSENSOR MATERIALS AND METHODS

Technical Field

This invention relates to biosensor materials and methods, and in particular to methods for generating microorganisms having utility in biosensing, tools which can be generally used in such methods, the microorganisms themselves, and biosensing methods employing such microorganisms.

Background Art

It is frequently desirable to be able to detect small concentrations of analytes in samples, e.g. environmental samples. For instance, to allow more effective management of scarce environmental resources, more efficient and faster methods of assessing environmental pollution are required. At present, molecular-specific monitoring of effluent streams and other environmental matrices requires extensive chemical manipulation of the sample followed by Gas Chromatography (GC) and Mass Spectrometry (MS) analyses. Although these techniques are highly sensitive, sample preparation is necessarily slow and expensive. Consequently, continuous on-site analysis of a variety of environmental matrices cannot be achieved using these methods at reasonable cost.

A metal or xenobiotic sensor based on *E. coli* and genetic material from *Pseudomonas alkaligenes* has been proposed in WO 92/15687 (VITO). This employed previously characterised metal resistance genes.

An alternative method for the determination of phenols and chlorophenols has been proposed using a biosensor based around *Rhodococcus* sp. [see Riedel et al (1993) Appl Microbiol Biotechnol 38: 556-559]. In this method microorganisms are immobilised in an oxygen electrode, and oxygen uptake in response to added

substrates was monitored. Although fairly simple and rapid, this method lacks robustness and is not sufficiently sensitive or specific for detecting particular environmental pollutants.

Rhodococcus spp. have also been the subject of investigation for their potential in biocatalysis and bioremediation e.g. for the removal of sulphur from coal. For example Omori et al (1992) Applied and Environmental Microbiology: 911-915 investigated the intermediates and products formed in a particular pathway in the dibenzothiopene degradation process. Denome et al (1993) Applied and Environmental Microbiology 2837-2843, and Li et al (1996) J Bacteriol: 6409-6418 both studied the desulferization genes in *Rhodococcus erythropolis* (IGTS8), also using DBT. The nitrolase gene from *Rhodococcus rhodochrous* has also been studied in EP 0 759 474 (Shimizu/NITTO).

Figures

Figure 1 - shows an agarose gel on which digestions of the novel plasmid pRC100 has been run, as described in Example 5.

Figure 2 - shows an agarose gel on which digestions of the novel plasmid pRC158 has been run, as described in Example 5.

Figure 3 - shows a schematic view of the R. corallina ohp operon obtained by functional screening in E. coli, as described in Example 7. The schematic shows location of predicted genes: Regulator, Transport, Monooxygenase, Hydroxymuconic semialdehye hydrolase, Alcohol dehydrogenase. Initiator and terminator codons are shown as half height and full height lines respectively. Base coordinates refer to the Figure 4 sequence. The location of predicted promoter regions and direction are indicated by arrows. The molecular weights and coordinates of ohp genes are tabulated.

Figure 4 - shows the complete listing of the R. corallina ohp operon as described in Example 7. It includes a portion of a putative nitropropane promoter (5' of the regulator).

Figure 5 - shows a schematic diagram of the pJP8 vector of the present invention, as described in Example 8. Plasmid size is about 8.51 kb. pJP8 is a mycolic acid bacterium - E. coli mobilizable cosmid vector. It carries pCY104 replicon; is Kanamycin resistant 15 μg/ml mycolic acid bacteria, 50 μg/ml E. coli. It also carries lambda cos site, RP4 oriT site and a multiple cloning site.

Figure 6 - shows a schematic diagram of the pJP7 vector of the present invention, as described in Example 10. Plasmid size is about 10.66 kb. pJP7 is a mobilizable E. coli/Rhodococcus/Nocardia suicide/luciferase integration vector encoding luxAB signal genes, sacB gene

Claims

1. A method for identifying and/or isolating mycolic acid bacterial DNA encoding an inducible promoter which is induced in response to a specific analyte and/or associated operon proteins, the method comprising the steps of:
(a) culturing a source of mycolic acid bacteria in a selective medium containing said specific analyte and being selective for oligotrophic bacteria,
(b) identifying mycolic acid bacteria which are capable of subsisting on said medium and which lack catabolic repression,
(c) extracting DNA from said mycolic acid bacteria,
(d) incorporating said DNA into a vector,
(e) cloning said vector into a suitable host cell, and
(f) screening the host cell for said inducible promoter and/or proteins in order to identify vectors encoding it.

2. A method as claimed in claim 1 wherein the analyte is an environmental pollutant.

3. A method as claimed in claim 2 wherein the environmental pollutant is a hydrophobic organic compound.

4. A method as claimed in any one of the preceding claims wherein the mycolic acid bacterium is a member of the Rhodococcus or Nocardia complex.

5. A method as claimed in any one of the preceding claims wherein the medium used in step (a) comprises less than <500 uM carbon supplement.

6. A method as claimed in any one of the preceding claims wherein the catabolic repression screen is performed by assessing the concentration of an enzyme associated with the specific analyte of interest in (i) medium supplemented with the specific analyte, and (ii) medium supplemented with the specific analyte plus a high efficiency carbon source, and (iii) medium not containing the specific analyte but containing a high efficiency carbon source.

7. A method as claimed in any one of the preceding claims wherein the mycolic acid bacteria are grown on a medium comprising L-glycine prior to the DNA extraction at step (c).

8. A method as claimed in claim 7 wherein the mycolic acid bacteria are washed using 0.05 - 0.5 % (v/v) non-ionic detergent prior to the DNA extraction at step (c).

9. A method as claimed in any one of the preceding claims wherein the host cell of step (e) is an E coli strain carrying one or more of the mcrABC, mrr , hsdSRM recA or recC mutations.

AMENDED SHEET

10. A method as claimed in any one of the preceding claims wherein the host cell is screened for a sequence comprising an inducible promoter and/or operon proteins by using one or more oligonucleotide probes or primers corresponding to, or complementary to, a promoter and/or operon protein derived from a mycolic acid bacterium and selecting vectors which are complementary to, or specifically hybridisable with, said probe or primer.

11. A method as claimed in claim 10 wherein the oligonucleotide probe or primer comprises a sequence of at least 20, 30, 40, 50, or 100 nucleotides, said sequence corresponding to, or being complementary to, all or part of a contiguous sequence of the R. corallina ohp operon.

12. A method as claimed in any one of claims 1 to 9 wherein the host cell is screened by:
(i) incorporating a sequence believed to comprise an inducible promoter plus optionally further operon proteins in a vector at a position in which it is operatively linked to a coding sequence,
(ii) transforming a host cell with said vector, and
(iii) determining the presence or absence of the coding sequence expression product in the presence of the analyte.

13. As method as claimed in any one of claims 1 to 9 wherein the host cell is screened for the inducible promoter and/or operon proteins by screening for an activity associated with the inducible promoter and/or operon proteins.

14. A method as claimed in claim 13 wherein the activity is an enzyme activity for which the analyte is a substrate.

15. A method as claimed in claim 14 wherein the enzyme activity is screened for by contacting the host cell or an extract thereof with a substrate for the enzyme and observing the cell or extract for enzymatically generated products of the substrate.

16. A method as claimed in any one of claims 13 to 15 wherein the vector in transferred from a first host cell of step (e) to a second host cell wherein the activity is screened.

17. A method as claimed in claim 16 wherein the second host is a mycolic acid bacterium.

18. A method as claimed in claim 17 wherein the second host is a Corynebacterium.

19. A method as claimed in any one of claims 16 to 18 wherein the vector in transferred from the first to the second host by bacterial conjugation.

20. A method as claimed in any one of claims 16 to 19 wherein the vector is shuttle vector capable of replication in the first and second hosts.

21. A method as claimed in claim 20 wherein the vector comprises two, three, four or five of the following elements: (i) a replicon for mycolic acid bacteria; (ii) a replicon for E. coli; (iii) a conjugative origin of transfer; (iv) a lambda cos site; (v) a sequence encoding an antibiotic marker gene.

22. A method as claimed in claim 21 wherein the elements are selected from a group comprising: pCY104oriV ; pBR322 oriV; RP4 oriT; pSR1.

23. A method as claimed in claim 22 wherein the plasmid is selected from: pJ8; pRV1; pJH6 as described herein.

24. A method as claimed in any one of claims 21 to 23 wherein the vector comprises a lambda cos site, and sufficient DNA from the mycolic acid bacteria is incorporated into the vector to allow in vitro packaging of the vector.

25. A method of producing a modified inducible promoter and/or operon, the method comprising the step of modifying a nucleotide sequence encoding the inducible promoter and/or operon identified in accordance with the method of any of the preceding claims.

26. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an inducible promoter and/or operon protein identified in accordance with the method of any one of claims 1 to 24 or produced by the method of claim 25.

27. A nucleic acid as claimed in claim 26 comprising a promoter region of the nucleotide sequence encoding the R. corallina ohp operon described in Figure 3.

28. A nucleic acid as claimed in claim 26 encoding one or more of the following proteins of the R. corallina ohp operon: Regulator REG; Transport TRANS; Monooxygenase MONO; Hydroxymuconic semialdehye hydrolase HMSH; Alcohol dehydrogenase ADH; and Catechol 2, 3-dioxygenase CDO.

29. A nucleic acid molecule comprising a sequence encoding a modified inducible promoter obtainable by the method claim 25 which is at least 70%; 80%; 90%; 95% or 98% identical to the sequence of the inducible promoter of claim 26 or claim 27.

30. A nucleic acid as claimed in any one of claims 26 to 29 further comprising a heterologous signal gene.

31. A nucleic acid comprising (a) a sequence capable of effecting site specific integration of a heterologous signal gene into the genome of host cell such that it is operably

linked to an inducible promoter identified in accordance with the method of any one of claims 1 to 24; (b) a heterologous signal gene.

32. A vector comprising the nucleic acid of claim 30 or claim 31.

33. A vector as claimed in claim 32 comprising one or more of the following: luxAB signal genes; sacB gene; antibiotic resistance; RP4/RK2 mobilizing elements.

34. A vector as claimed in claim 33 which is pJP7 as described herein.

35. A method of transforming a host cell comprising use of a vector as claimed in any one of claims 32 to 34.

36. A method as claimed in claim 35 wherein the host cell is transformed by site specific integration such that the signal gene is operably linked to an endogenous inducible promoter.

37. A method as claimed in claim 35 or claim 36 wherein the host cell is a mycolic acid bacterium of the same strain from which the inducible promoter and/or operon proteins were isolated.

38. A method of producing a biosensor comprising the method of any one of claims 35 to 37.

39. A biosensor host transformed with a vector as claimed in any one of claims 32 to 34 or as produced by the method claim 38.

40. A method of detecting the presence or absence of an analyte in a sample comprising the steps of:
(a) contacting the sample with a transformed microorganism which is a mycolic acid bacterium which lacks catabolic repression and which expresses a binding agent capable of binding the analyte, wherein the binding of the agent to the analyte causes a detectable signal, and wherein said bacterium has been transformed such as to improve the detectability of the signal; and
(b) observing said bacterium for said detectable signal.

41. A method as claimed in claim 40 wherein the transformed microorganism is the biosensor of claim 39.

42. A method as claimed in claim 40 or claim 41 wherein the signal is detected by an increased expression of a heterologous signal protein from a signal gene.

43. A method as claimed in any one of claims 40 to 42 wherein the signal is detected photometrically.

44. A kit for performing the method of any one of claims 40

to 43 comprising (a) a biosensor as claimed in claim 39, plus (b) one or more further materials for performing the method.

45. A kit for performing the method of any one of claims 1 to 24 comprising two or more of the following (a) the selective buffer of claim 5; (b) a non-ionic detergent; (c) the primers or probes of claim 12; (c) the vector of any one of claims 21 to 24.18 August 1999

AMENDED SHEET